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THE EFFECT OF VITAMIN E DEFICIENCY UPON INTRAUTERINE DEVELOPMENT AND THE CHANGES IN THE MAMMARY GLAND OF THE ALBINO RAT

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA

BY

JOHN ARNOLD URNER

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
JUNE 1929

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THE CHANGES IN THE MAMMARY GLANDS OF THE PREGNANT ALBINO RAT DEPRIVED OF VITAMIN E*

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JOHN A. URNER, M.D. PH.D.
MINNEAPOLIS

The discovery of the fat soluble, antisterility vitamin E, by Evans and Bishop in 1922, stimulated great interest and much research on the phenomena of reproduction in the absence of this important dietary constituent.

All observers who have investigated the reproductive behavior of animals maintained on highly purified diets have also noted interference with lactation.

Evans and Burr [1] observed paralysis of the hind legs of suckling rats whose mothers had been fed a diet with only sufficient vitamin E to permit a successful termination of pregnancy. This paralysis was prevented prophylactically by feeding the mother larger doses of vitamin E than was necessary for the function of reproduction alone.

While these disturbances are no doubt chemical in nature, it is of fundamental importance to determine any anatomic change that may underlie this disturbed function.

I wish to express my thanks and appreciation to Dr. Jennings C. Litzenberg, Dr. C. M. Jackson, and Dr. George O. Burr for their advice and criticism during the course of this study.

* From the Department of Obstetrics and Gynecology and Anatomy, University of Minnesota Medical School.

* Read before the Section on Obstetrics, Gynecology and Abdominal Surgery at the Eighty-First Annual Session of the American Medical Association, June 25, 1930.

1. Evans, H. M., and Burr, G. O.: Development of Paralysis in the Suckling Young of Mothers Deprived of Vitamin E, J. Biol. Chem. **76**: 273-297, 1927. The Antisterility Vitamin, Fat Soluble E. Memoirs of the University of California **8**, 1927.

MATERIAL AND METHODS

The material used in this investigation consisted of the mammary glands removed from eighty-three pregnant albino rats.

The animals were reared on a diet deficient in vitamin E (561 Burr), composed of casein (crude) 60.0, sucrose 90.0, salts (185, McCollum) 9.0, and lard 40.0. In addition, each animal was individually fed 0.6 Gm. of powdered yeast and three drops of cod liver oil daily.

All animals were bred at approximately 90 days of age and without exception were found to be relatively sterile and showed resorption of their pregnancies, characteristic of vitamin E deficiency.

Forty-three animals were continued on the vitamin E deficient diet while forty were changed to a curative diet, which differed from the foregoing in that 0.2 Gm. of powdered yeast and 0.4 Gm. of wheat germ were given daily, beginning at the date of the second breeding.

Animals of both groups were killed from the seventh to the twenty-second day of pregnancy inclusive; the cured animals served as controls for those deficient in vitamin E.

The skins were removed with the mammary glands attached, after the method described by Maeder.[2] Blocks of glandular tissue were prepared for cleared preparations by the method of F. J. and J. A. Myers.[3] Tissue for microscopic examination was fixed in Zenker's solution, embedded in paraffin, sectioned at 10 microns and stained in hematoxylin and eosin by the routine method of tissue preparation.

This study consisted of observations of the gross glands attached to the skin, examination of cleared preparations and a detailed histologic study. The size of the acini, the height of the acinous cell and the diameter of the acinous lumens were measured with a filar micrometer. The average of twenty measurements was recorded for each element in each section studied.

2. Maeder, L. M. A.: Changes in the Mammary Gland of the Albino Rat During Lactation and Involution, Am. J. Anat. **31**: 1-26, 1922.

3. Myers, F. J., and Myers, J. A.: Studies on the Mammary Gland: VIII. Gross Changes in the Mammary Gland in the Female Albino Rat During the Period of Involution, Proc. A. Anat., Anat. Rec. **21**: 74, 1921.

OBSERVATIONS

When the living animal is examined during pregnancy, the only change observed is a slight enlargement and increase in length of the nipple. This has been shown by Myers [4] to be due to the complete degeneration of the epithelial hood, which began but was only partially completed at the period of puberty.

During the first week following insemination there is very little change until the fourteenth day, when the nipple appears to be definitely elongated.

The examination of the glands attached to the skin reveals two U shaped accumulations of glandular tissue and fat, one in the thoracic region and the other in the inguinal and lumbar regions. During the first week of pregnancy there is slight change in the character of these areas, but by the fourteenth day there is steady increase in size until the eighteenth or nineteenth day, after which there is progressive diminution in size and thickness. The glands in control animals examined under the same conditions show progressive increase in size and thickness up to the twenty-second day of pregnancy.

CLEARED PREPARATIONS

Low power microscopic examination of cleared preparations showed that during the first half of pregnancy there was a steady increase in the number of ducts and lobules, which by the eighteenth or nineteenth day became greatly distended and during the subsequent days collapsed and disappeared from large areas of the sections examined. In the control animals on an adequate vitamin E diet, the glands showed a gradual increase in the size of the lobules up to the end of the period of observation.

HISTOLOGIC CHANGES

The histologic examination, beginning with the seventh day of pregnancy, showed an increase in the connective tissue stroma surrounding the ducts, alveoli and lobules, in which newly formed circulatory structures were found.

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4. Myers, J. A.: Studies on the Mammary Gland: I. The Growth and Distribution of the Milk Ducts and the Development of the Nipple in the Albino Rat from Birth to Ten Weeks of Age, Am. J. Anat. **19**: 353-389, 1916; Fetal Development of the Mammary Glands in the Female Albino Rat, ibid. **22**: 195-223, 1917.

The ducts increased in number, from which large numbers of alveoli were seen as budding outgrowths pushing a layer of stroma before them.

Vacuoles appeared in the cytoplasm of the acinar cells by the twelfth day of pregnancy and in the cells lining the ducts by the fourteenth day.

Measurements of the glandular elements during this period showed little or no change in their size, thus indicating that this development is one of simple hyperplasia.

These observations agree with those of Roberts [5] and are comparable to the changes observed in the cured control glands.

By the fifteenth day of pregnancy one out of three glands showed signs of involution, in which collapsed and necrotic acini and connective tissue casts of absorbed acini and ducts were visible. The remaining glands showed greater secretory activity, as evidenced by an increase in the size of the acini and in the greater number of secretion droplets in the acinous cells.

During the subsequent days there was, with some variation, a general increase in the size of the glandular elements and still greater accumulation of secretion droplets. The acinar lumens and the ducts became filled with secretion. Scattered areas in the tissue examined showed collapsed acini and ducts undergoing degeneration.

At the eighteenth day, every gland gave the appearance of a lactating stage in which the acini and ducts were enormously distended with secretion. The secretion droplets in the acinar cells were greatly reduced in numbers. In large areas the cell walls were collapsed and dark in appearance, while in others there was degeneration and dissociation of acini and the smaller ducts.

The nineteenth and twentieth day sections showed that, after the distention of the acini and ducts and the loss of secretion droplets from the cell, there is rupture of the cell wall and degeneration of the nucleus and cellular remains. This produces a relative connective tissue increase, and in many areas connective tissue casts are seen from which the epithelial elements have been absorbed.

5. Roberts, F. L.: Changes in the Mammary Glands of the Albino Rat During the Second Half of Pregnancy, the Graduate School of the University of Minnesota, Papers from the Mayo Foundation for Medical Education and Research, Philadelphia, W. B. Saunders Company **1**, 1921.

Examination of the stages at the twenty-first and twenty-second days showed a variable picture. In general it was one of almost complete involution, as evidenced by small islands of glandular tissue and the presence of connective tissue casts. The larger ducts contained secretion.

The control glands examined from the fourteenth until the twenty-second day showed beginning secretory activity by the formation of secretion droplets in the acinar and duct cells by the fourteenth day. The height of the acinar cells became increasingly smaller and the diameter of the acinar lumens larger during the subsequent days, reaching its maximum size between the twenty-first and twenty-second days. This increase is due to the accumulation of secretion in preparation for lactation.

COMMENT

In addition to these observations on the mammary gland, I have repeated the work of Evans, Burr and Althausen on the histopathology of gestation in the rat deprived of vitamin E.

The correlation of these studies is of fundamental importance.

My observation showed that during the seventh, eighth and ninth days of pregnancy the growth of the embryo appeared to be normal. By the tenth day of gestation there was evidence of failure of the mesodermal derivatives of the embryo, most marked in the hematopoietic structures. The embryo and its membranes rapidly dissociated and disappeared by the fourteenth day of pregnancy.

The placenta began to show degenerative changes in its mesodermal structures by the twelfth day. These changes were cellular necrosis, obliteration of the sinus relationship between the fetal and maternal circulation, and progressive destruction proceeding toward the decidua basalis.

All identification of the placenta and decidua basalis was destroyed by the fifteenth day, at which time they were found as a mass of necrosing cells surrounded by large ectodermal giant cells in syncytial arrangement.

The necrotic remains of the placenta and decidua basalis remained attached to the uterine wall until the eighteenth or nineteenth day, when they became completely dissociated and were found in the uterine lumen as cellular débris mixed with blood.

It will be noted that until the fourteenth day of pregnancy the mammary glands of the vitamin E deficient animals were comparable to their cured controls and those from animals on stock diets studied by Roberts. During this time there had been implantation and development of normal embryos until the tenth day, failure of the fetal mesoderm resulting in destruction of the embryo and advanced degeneration of the placenta.

The involution changes observed at the fifteenth and subsequent days correspond in time to the advanced degeneration and destruction of the placenta and decidua basalis and their separation from the uterine wall.

That the embryo did not carry the mammary gland into an advanced secretory stage is shown by its disappearance by the fourteenth day.

It is probable that the stimulation over a period of four days was continued by the remaining placenta and decidua, which, when completely destroyed, permitted the gland to go into rapid involution.

A similar condition has been found by Loeb and Hesselberg [6] in abortion experiments on guinea-pigs.

SUMMARY AND CONCLUSIONS

1. Gross observation of the mammary glands of forty-three pregnant albino rats deprived of vitamin E showed normal growth and development until the eighteenth or nineteenth day, at which time there was marked diminution in size of the masses of tissue.

2. The observation of cleared preparations showed normal growth and development until the eighteenth day of pregnancy, when the ducts and lobules were greatly distended and during the later days collapsed and diminished in size.

3. The histologic study showed normal development until the fifteenth day, at which time involution changes began and progressed rapidly until the end of the period of observation.

4. Involution changes in the mammary glands followed the advanced degeneration of the placenta and

6. Loeb, Leo; and Hesselberg, Cora: The Cyclic Changes in the Mammary Gland Under Normal and Pathological Conditions: 1. Changes in the Nonpregnant Guinea-Pig; 2. The Changes in the Pregnant Guinea-Pig. J. Exper. Med. **25**: 285-322, 1917.

decidua basalis, which is characteristic of vitamin E deficiency on pregnancy in the rat.

5. Therefore involution changes in the mammary gland are apparently due to vitamin E deficiency secondary to the death of the embryo and the consequent degeneration in the membranes, placenta, and decidual tissue.

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Reprinted from The Anatomical Record
Vol. 50, No. 2, August, 1931

THE INTRA-UTERINE CHANGES IN THE PREGNANT ALBINO RAT (MUS NORVEGICUS) DEPRIVED OF VITAMIN E

JOHN A. URNER

Departments of Obstetrics and Gynecology and of Anatomy, University of Minnesota, Minneapolis, Minnesota

TWO FIGURES

INTRODUCTION

The recent work of Evans, Burr, and Althausen ('27) upon the histopathology of gestation in the rat deprived of vitamin E is of sufficient importance to justify further investigation. This study, therefore, was undertaken in order to extend our knowledge of this deficiency. I wish to thank Dr. Jennings C. Litzenberg, Dr. C. M. Jackson, and Dr. George O. Burr for their advice and criticism during the course of this investigation.

MATERIAL AND METHODS

The material consisted of ninety-one female albino rats, bred from normal adult Wistar stock and reared in the animal colony of the Department of Anatomy, University of Minnesota. The young rats were weaned on their twenty-first day of life and were given a basal diet (Burr no. 561) composed of: casein (crude), 30.0; sucrose, 45.0; salts (McCollum no. 185), 4.5, and lard, 20.0. In addition, each animal was individually fed 0.6 gram of powdered yeast and three drops of cod-liver oil each day. The rats were weighed at five-day intervals, and, when fifty days of age, vaginal smears were taken to establish their estrous behavior.

The first breeding was carried out at seventy-five days of age. A positive breeding was recorded when sperm were

found in the vagina after twelve hours with the male. No attempt was made to time the gestations more accurately than twelve hours. Positive pregnancy was diagnosed by the appearance of the 'red-blood-cell sign' on the fourteenth or fifteenth day following insemination. After the appearance of this sign, daily weights were recorded in order to detect resorbing pregnancies.

After the initial breeding, which terminated by the resorption of the pregnancies in every animal and determined their relative sterility, the rats were divided into two groups. One group of forty-three rats was continued upon the vitamin-E-deficient diet. The other forty-seven rats were given a curative diet, which differed from the foregoing in that, in addition to the basal diet, 0.2 gram of powdered yeast and 0.4 gram of wheat germ and three drops of cod-liver oil were fed each day, beginning with the date of the second breeding.

All animals were rebred to obtain material for study. Beginning with the seventh day of pregnancy, rats on the vitamin-E-deficient diet and those on the curative diet were killed at each successive day. The 'cured' animals served as controls. Six rats were used for each day, from the tenth to the seventeenth days, inclusive, of which three were on the E-deficient and three on the curative diet. Two rats were used for gross examination and four for microscopic study. On the seventh, eighth, ninth, and from the eighteenth to the twenty-first day, inclusive, four rats were used, of which two were vitamin-E deficient and two were 'cured' controls. One of each group was utilized for gross examination and the other for microscopic study.

The preparation of material for microscopic examination consisted in the removal of the largest embryos with a corresponding segment of the uterus. These were fixed in Bouin's and Zenker's fluid and embedded in paraffin by the usual methods. The early specimens were sectioned and mounted serially; in the larger ones, every tenth section was mounted. Hematoxylin and eosin were used to stain all sections.

THE CHARACTERISTICS OF THE ALBINO RAT DEPRIVED OF VITAMIN E

Some general observations on the E-deficient rats used in this investigation will be summarized briefly.

The growth curve of the group, as a whole, is shown in figure 1. As will be seen, it is entirely satisfactory and

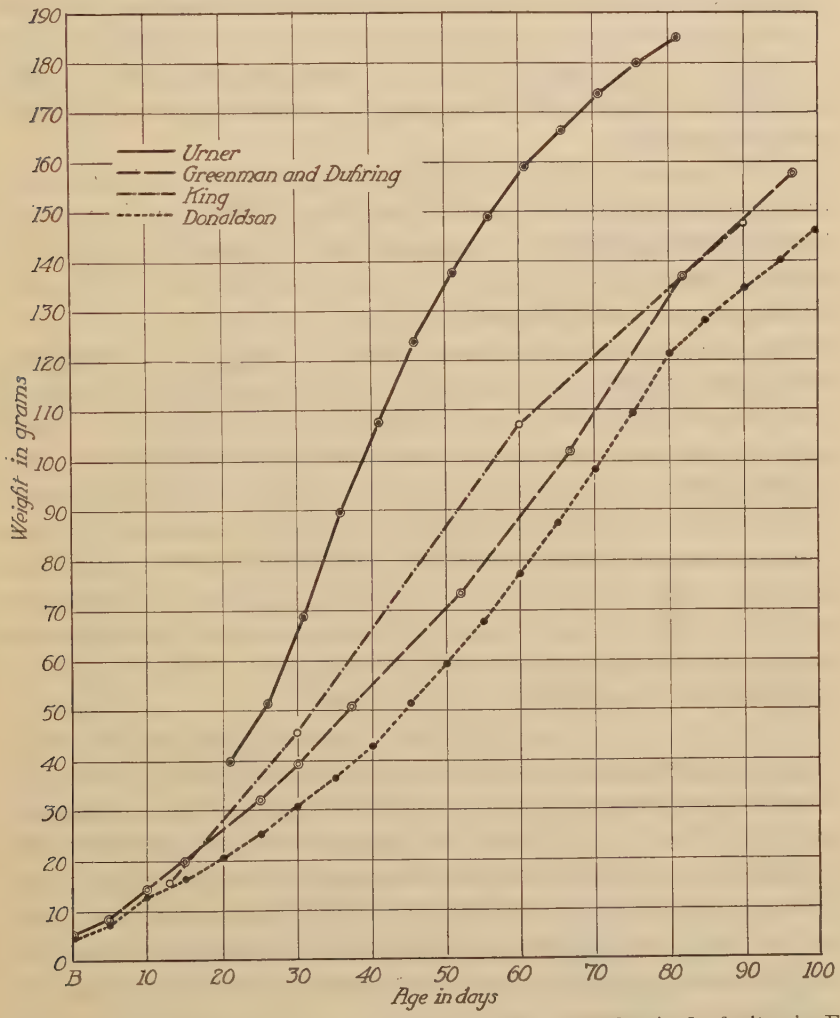

Fig. 1 Average growth curve of ninety-one albino rats deprived of vitamin E (Urner) compared with similar curves of animals reared on stock diets.

superior to those of normal stock rats of the same strain, according to data published by Donaldson ('24).

The entire group of rats exhibited no nutritional defects aside from those associated with the deficiency of vitamin E. They were remarkably free from infection, as revealed by careful necropsy.

The estrous rhythm was found to be very regular, as 89.6 per cent of the cycles fell within the four-, five-, and six-day intervals. Long and Evans ('22) observed that only 82 per cent of the cycles of their stock animals occurred during these intervals.

The breeding records show that there were 77.4 per cent positive inseminations and 5.2 per cent failures of implantation following positive insemination—a figure well within normal limits, as shown by Evans and Bishop ('23) and Evans, Burr, and Althausen ('27).

The weight curve during pregnancy (fig. 2) is compared with that from Evans, Burr, and Althausen. My curve shows that, especially during the first ten days of pregnancy, there was normal gain, due to the development of the embryos, the hypertrophy of the mammary glands, and probably the stimulation of the general metabolism due to pregnancy. From the tenth until the fifteenth day, retardation of the increase in body weight was apparent. During this time the embryos underwent death and partial resorption and the placenta became markedly degenerated. The mammary glands showed progressive hypertrophy (Urner, '30).

There are thus two opposing factors affecting the body weight at this time: loss due to fetal and placental degeneration, and the gain due to the development of the mammary glands. The latter factor is apparently predominant and probably produces the slight average gain during this time. This gain continues from the fourteenth to the sixteenth day, while there is still more destruction of the placentae and uterine decidua and the mammary glands are developing into the secretory stage.

From the sixteenth until the eighteenth day, there is almost complete resorption of the products of conception and beginning involution of the mammary glands. The body weight now begins to decrease, as shown in figure 2. From the eighteenth to the twenty-second day, there is marked involution of the mammary glands, which seems to account for the greater loss in body weight during this period (Urner, '30).

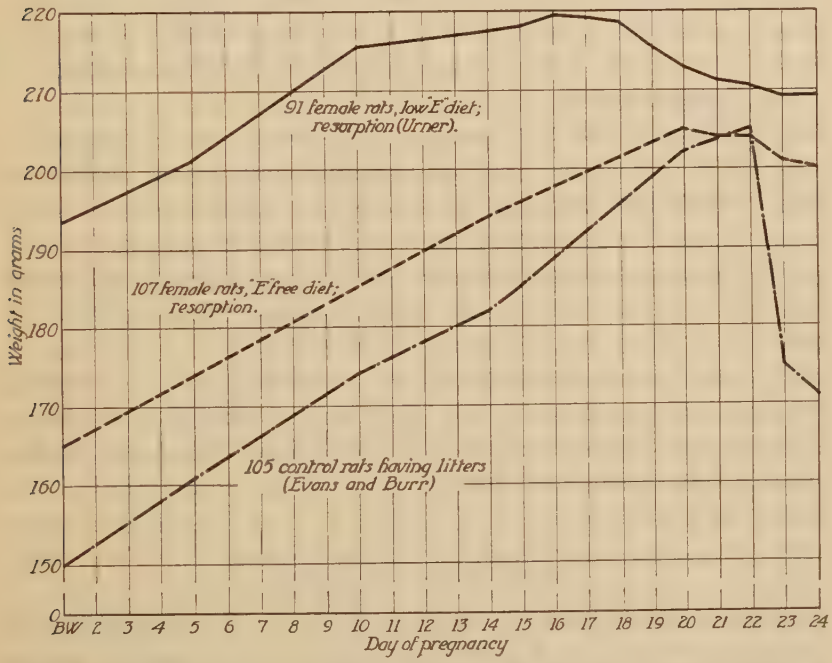
220
210
200
190
180
170
160
150
0
Weight in grams
91 female rats, low E diet; resorption (Urner).
107 female rats, E free diet; resorption.
105 control rats having litters (Evans and Burr)
BW 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Day of pregnancy

Fig. 2 Curve showing the average weights of ninety-one rats deprived of vitamin E from the day of breeding to the twenty-fourth day of pregnancy, compared with similar curves from Evans, Burr, and Althausen. *BW* indicates breeding weight.

GROSS EXAMINATION OF THE PREGNANT UTERI AND CONTENTS REMOVED FROM RATS DEPRIVED OF VITAMIN E

Gross examination of uteri removed from E-deficient rats on the eighth and ninth days of pregnancy showed the beginning purple discoloration and congestion of the mesometrial vessels, characteristic of normal pregnancy. There were no

abnormalities observed on these days when compared with control uteri in rats on the curative diet.

By the tenth day, a few of the enlargements of pregnancy developed a distinct bluish color, in marked contrast to the purple color of normal pregnancy. These enlargements were softer to palpation and, when dissected free from the uterus, showed a blood-tinged amniotic fluid, an embryo appearing grossly normal, and free blood surrounding the blastocyst. From the tenth to the twelfth day, inclusive, there were occasional apparently normal enlargements, which, when dissected free from the uterus, invariably showed a slightly blood-tinged amniotic fluid.

After the twelfth day, there was never a normal-appearing pregnancy. All the uterine enlargements were definitely blue in color and softened in consistency. Embryos were not grossly visible after this time. The enlargements subsequently became more fusiform in shape and by the fifteenth day assumed a bluish-black color. When opened, they revealed only dark blood-stained débris.

The uteri involuted rapidly after the fifteenth day and at the end of the twenty-first day had assumed an almost virginal condition, except for the small decidual bodies (Duval, '91) at the mesometrial border, in the location of the recent implantations.

MICROSCOPIC CHANGES

Examination of serial sections through the enlargements of pregnancy in the E-deficient rats, on the seventh day of pregnancy, showed an apparently normal decidual cavity. The distal entodermal layer separated the blood-filled decidual cavity from that of the blastocyst. When compared with the seventh-day stage in the 'cured' controls, these embryos appeared entirely normal and corresponded to this stage of gestation, described by Duval ('91) and Huber ('15).

The eighth-day sections revealed the ectodermal and amniotic cavities separated by the inter-amnio-placental fold and the mesoderm making its appearance between the entoderm

and ectoderm. There was blood in the decidual cavity, but it made no contact with the embryo. This stage of gestation appeared entirely normal when compared with the controls, but was somewhat more advanced than the eighth-day stages described by Duval and Huber.

The ninth-day sections showed the development of the amniotic and ectoplacental cavities. The entire blastodermic sac was surrounded by maternal blood which did not penetrate into the amniotic cavity. There were no abnormalities observed in these embryos and they compared well with the same stages for the normal animal.

The tenth day of gestation in the E-deficient rat revealed beginning rarefaction of the mesenchymal tissues of the embryo. The heart contained a few scattered fetal blood cells. The yolk sac showed a few small blood islands. The layers of the ectodermal cavity had fused to form the ectoplacenta. The allantois had approached the inferior layer of the ectoplacenta in one embryo and had fused with it in another. The ectoplacenta was composed of an inferior or compact cellular layer and a plasmodial layer between which were wide maternal sinuses. Superiorly, the ectoplacental cone was seen approaching the mesometrial portion of the uterus. Control embryos examined on this day showed more advanced development in that the inferior layer of the ectoplacenta (with which the allantois had fused) was penetrated by mesodermal buds containing fetal blood cells; thus the establishment of circulation between mother and embryo had taken place.

Embryos of the test rats examined on the eleventh day of gestation showed a general rarefaction of the mesenchymal tissue. The heart contained a few fetal blood cells and the liver had developed sinusoids, but showed a scarcity of fetal blood cells. The blood vessels were wide and contained very few fetal blood cells. The yolk sac and blood islands were poorly developed and contained a few fetal blood cells. The inferior layer of the ectoplacenta showed the allantois, spongy in appearance and greatly rarefied. The compact cellular

layer had been penetrated by a few mesodermal buds which were also rarefied in appearance. The plasmodial layer was occupied by very large maternal sinuses, while the superior layer was composed of clear vesicular cells and numerous ectodermal giant cells—the remains of the ectoplacental cone.

The twelfth-day embryos of the test rats presented the same general picture as those of the previous day. The amniotic cavity now contained free blood. There was extreme rarefaction of the embryonic structures and large portions were undergoing degeneration, as evidenced by pycnotic nuclei and disintegration of the cell boundaries. There was more advanced degeneration of the mesodermal elements of the ectoplacenta. The plasmodial layer was flat and showed great numbers of giant cells scattered throughout.

By the thirteenth day, the embryo had degenerated almost entirely. The fetal surface of the ectoplacenta had undergone necrotic changes, beginning in the areas that had been occupied by mesoderm. This was followed by rupture of the cells and casting off of the degenerated portions into the amniotic cavity. The superior portions of the ectoplacenta continued to develop by the conversion of decidual cells into clear vesicular cells which were surrounded by large numbers of ectodermal giant cells.

The fourteenth day showed complete destruction of the embryo, which remained only as cellular débris. The fetal surface of the ectoplacenta was greatly dissociated. There were no definable layers of the ectoplacenta, as the maternal sinuses had ruptured and had lost their relationship. The deeper layers of the decidua basalis were developing, as in the previous day, by conversion into clear vesicular cells.

By the fifteenth day, still more of the ectoplacenta was destroyed. The remaining portion showed invagination into the uterine lumen. The lateral margins had degenerated as far as the circular muscular layers of the uterus. The entire remaining portion of the uterine decidua appeared necrotic.

During the fifteenth, sixteenth, and seventeenth days the necrotic remains of the ectoplacenta were separated from the

uterine wall and were cast off into the uterine lumen. The placental site was covered over with uterine mucosa and the uterus had returned to an almost virginal condition, excepting the decidual bodies at the mesometrial border.

DISCUSSION

The uteri removed from the vitamin-E-deficient rats, on the eighth, ninth, and tenth days of pregnancy, presented an appearance similar to those of 'cured' controls examined at the same period of gestation. As early as the tenth day, the enlargements developed the external blue color, due to the blood-stained amniotic fluid. In the subsequent days there was no doubt that every enlargement was abnormal, as it contained blood in the amniotic cavity and exhibited unquestionable signs of degeneration.

Examination of the uteri of the 'cured' control group also showed many enlargements possessing the blue discoloration, although some of the implantations appeared normal. It should be remembered that a small proportion of resorptions occur even during the normal pregnancy in the rat.

From the microscopic observations of the pregnancies in the E-deficient rats it is evident that there is complete degeneration and resorption—in general agreement with the observations of Evans, Burr, and Althausen.

During the seventh, eighth, and ninth days, the stages in my test rats were comparable in development to the 'cured' controls at the same period of pregnancy, as well as to similar stages described by Duval ('91) and Huber ('15) for the normal animal. Evans, Burr, and Althausen have described retarded development of the embryos in E-deficient rats during this early period, but this is contrary to my observations. However, my tenth-day embryos corresponded to the early eleventh-day stages described by Duval, as they showed normal development of the ectoplacenta, fusion of the allantois to its base, and the beginning of maternal and fetal circulation.

Free blood in the uterine lumen was described by Evans, Burr, and Althausen. This has likewise been noted by Huber, in uteri possessing an abnormal endometrium, in which maternal blood and leucocytes were in direct contact with the ovum. I have also observed this condition, although the blood did not make contact with the embryo until the later days of pregnancy, when there were degenerative changes in the embryo.

Blood in the decidual cavity, when outside of the ectoplacental cone and the ectodermal layer of giant cells surrounding the egg, is not to be considered abnormal, as it was found in the vitamin-E-deficient and the control animals alike. Duval and Huber also noted this occurrence during the normal implantation in the rat.

My earliest evidence of embryonic pathology is on the tenth day, in which there is beginning deficiency of the mesoderm. I am unable to find any abnormal development of the ectoplacenta, as it corresponds to the normal stages for this period.

On the eleventh day, the embryo shows signs of a deficient blood-forming mechanism. The ectoplacenta shows retardation in its differentiation, as the mesodermal portions are not only poorly developed, but show rarefaction. The compact cellular layer is barely pierced by mesodermal buds. The superior layers show large numbers of clear vesicular cells and an abnormal number of giant cells—the remains of the ectoplacental cone. This observation was made also by Evans, Burr, and Althausen. The abnormality of the blood-forming tissue is thus associated with the failure of the mesoderm to penetrate the compact cellular layer of the ectoplacenta and to displace the remaining cells of the ectoplacental cone as occurs in normal placental development.

In my observations, fetal death, based upon signs of definite disintegration, is certain to have occurred on the twelfth day. By the thirteenth day, in most cases, the embryo had almost completely disappeared.

The ectoplacenta continued in the development of the superior portions by the conversion of decidual cells into normal-appearing vesicular cells as in normal development, while the fetal surface dissociated progressively toward the decidua basalis. Finally, the necrotic remains of the ectoplacenta were cast into the uterine lumen, where they were absorbed.

It will be noted that, according to my observations, the stages described are rather clearly differentiated. In general, the pathological changes agree with Evans, Burr, and Althausen. I have been able to confirm their observations as to changes in the mesodermal elements of the embryo and that the changes in the ectoplacenta are the result of failure of the mesoderm. My finding that the decidua continues in apparently normal development is also in agreement with their observations.

The rate of destruction of the embryos in this study was more rapid than that observed by Evans, Burr, and Althausen. I was unable to find an intact embryo after the twelfth day of pregnancy, while they found them remaining in various stages of dissociation until the later days of the gestation period. This may be attributed to a greater deficiency of vitamin E in the diet used in the present study. It will be noted that in my rats there was no first-litter fertility, such as was frequently found by Evans, Burr, and Althausen. These discrepancies might also be due to the marked differences between the Wistar albino and the Long-Evans hybrid strain of rats, as recently described by Freudenberger ('31).

SUMMARY AND CONCLUSIONS

Ninety-one Wistar albino rats, deprived of vitamin E and used in this investigation, exhibited normal growth, estrous rhythm, and breeding behavior.

Pregnancy in the entire group of animals was followed by embryonic death and resorption. No first-litter fertility was observed.

From the tenth to the sixteenth day of pregnancy, the embryonic and fetal resorption is more than counterbalanced by continued mammary development, so that the body weight continues to increase, although at a reduced rate. After the sixteenth day, the body weight rapidly decreases, associated with mammary involution.

Gross examination of the pregnant uteri (from the tenth day onward) showed a softening of the enlargements, with a blue discoloration, due to blood in the amniotic cavity. These indicate abnormal pregnancy.

Microscopic examination of serial sections of embryos showed apparently normal embryonic development until the tenth day of pregnancy. At this time there appear a rarefaction of the mesenchyme, failure of the blood-forming tissues, and death of the embryo.

The changes in the ectoplacenta became evident by the tenth day of gestation in the more compact appearance of the fetal portion and the failure of the mesodermal elements. Necrosis began at the fetal surface by the thirteenth day and progressed toward the mesometrial portion. It was entirely destroyed and cast into the uterine lumen by the seventeenth or eighteenth day.

The decidua basalis retained its normal appearance and became converted into tissue which would form the maternal portion of the normal ectoplacenta until it was overtaken by degenerative changes on the fifteenth day.

By the end of the twenty-first day of pregnancy, the uterus has returned to an almost virginal condition, with the exception of the decidual bodies at the site of the recent implantations.

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VITA

Author—John Arnold Urner.

Born—Joliet, Montana, September 20, 1895.

Academic History—Graduated from the Broadway High School, Seattle, Washington, in 1915. B.S. 1922, M.D. 1924, University of Minnesota. Entered the Graduate School, University of Minnesota, January 1926; received the Ph.D. degree in June 1929, major Obstetrics and Gynecology, minor Anatomy.

Present Position—Assistant Professor of Obstetrics and Gynecology, University of Minnesota, 1929—; Obstetrician and Gynecologist-in-Chief, The General Hospital, Minneapolis, Minnesota, 1929—.